09/749,185

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     (FILE 'HOME' ENTERED AT 08:47:19 ON 02 DEC 2004)

     INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE,
     AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS,
     BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB,
     CROPU, DDFB, DDFU, DGENE, DISSABS, ...' ENTERED AT 08:58:24 ON 02 DEC 2004
               SEA SACCHAROPOLYSPORA
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               2   FILE ADISCTI
               1   FILE ADISINSIGHT
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               1   FILE ANTE
              54   FILE BIOBUSINESS
               4   FILE BIOCOMMERCE
             132   FILE BIOENG
             463   FILE BIOSIS
             287   FILE BIOTECHABS
             287   FILE BIOTECHDS
             261   FILE BIOTECHNO
             201   FILE CABA
              23   FILE CANCERLIT
             612   FILE CAPLUS
              79   FILE CEABA-VTB
               3   FILE CEN
               6   FILE CIN
              13   FILE CONFSCI
              18   FILE CROPU
               5   FILE DDFB
              36   FILE DDFU
             516   FILE DGENE
              14   FILE DISSABS
               5   FILE DRUGB
              36   FILE DRUGU
               5   FILE EMBAL
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             186   FILE ESBIOBASE
               4   FILE FEDRIP
              10   FILE FROSTI
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             350   FILE GENBANK
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              38   FILE JICST-EPLUS
             262   FILE LIFESCI
             311   FILE MEDLINE
               7   FILE NIOSHTIC
               1   FILE NTIS
             265   FILE PASCAL
               6   FILE PHAR
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              12   FILE PROUSDDR
             651   FILE SCISEARCH
               1   FILE SYNTHLINE
             138   FILE TOXCENTER
             434   FILE USPATFULL
              43   FILE USPAT2
             102   FILE WPIDS
             102   FILE WPINDEX
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L1              QUE SACCHAROPOLYSPORA
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     FILE 'SCISEARCH, CAPLUS, BIOSIS, MEDLINE, EMBASE, BIOTECHDS, PASCAL,
     LIFESCI, BIOTECHNO, CABA' ENTERED AT 09:00:04 ON 02 DEC 2004
L2              2 S L1 AND SSGA
L3              2 DUP REM L2 (0 DUPLICATES REMOVED)
L4             18 S L1 AND SPORU?
L5              9 DUP REM L4 (9 DUPLICATES REMOVED)
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=> d l5 ibib ab 1-9

L5 ANSWER 1 OF 9 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-15969 BIOTECHDS
TITLE: Producing bioactive compound e.g. polyketide, by transforming host cells lacking bioactivity but possessing enzymic activity, with nucleic acid to provide second enzymic activity, and screening for bioactivity; bioactive compound production via plasmid expression in host cell for use in disease therapy
AUTHOR: KENDREW S G; PETKOVIC H; LEADLAY P F; MCARTHUR H A I
PATENT ASSIGNEE: BIOTICA TECHNOLOGY LTD; PFIZER INC
PATENT INFO: WO 2003033699 24 Apr 2003
APPLICATION INFO: WO 2002-GB4695 17 Oct 2002
PRIORITY INFO: GB 2001-25043 17 Oct 2001; GB 2001-25043 17 Oct 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-403221 [38]
AB DERWENT ABSTRACT:

NOVELTY - Producing (M) a bioactive compound having a known bioactivity, comprising providing host cells (HC) which substantially lack bioactivity but possess a first enzymic activity or a nucleic acid encoding an enzyme functional in a pathway leading to a compound having such bioactivity, introducing into HC a nucleic acid expressible in HC to provide a second enzymic activity, and screening for the bioactivity, is new.

DETAILED DESCRIPTION - Producing (M) a bioactive compound having a known bioactivity, involves providing host cells which substantially lack the bioactivity but possess at least one first enzymic activity or nucleic acid encoding at least one enzyme which is functional in a pathway leading to a compound having such bioactivity, introducing into the host cells nucleic acid expressible in the host cells to provide at least one second enzymic activity, where the first and second activities together enable the transformed host cells to produce a compound potentially having a known bioactivity, and screening for the bioactivity. An INDEPENDENT CLAIM is also included for recombinant cells (I) produced by introducing nucleic acids into host cells which substantially lack a predetermined bioactivity but posses at least one first enzymatic activity or nucleic acid encoding at least one enzyme which is functional in a pathway leading to such bioactivity, where the introduced nucleic acids are expressible in the host cells to provide at least one second enzymatic activity, where the first and second activities together enable the host cells to produce a compound potentially having a known bioactivity.

WIDER DISCLOSURE - Disclosed are: (1) a novel bioactive product obtainable by (M); (2) a vector system suitable for performing (M); (3) producing a library and screening for one or more desired bioactivities; (4) a method of making changes to a polyketide chain by manipulating the genes responsible for its production; (5) host strains specifically deleted of whole open reading frames of a polyketide synthase (PKS); (6) analyzing the effect of moving a PKS from one host to another; (7) rapidly screening randomly produced PKS enzymes; and (8) generating a library of bioactivities.

BIOTECHNOLOGY - Preferred Method: The bioactivity is screened on a culture of whole cells, or on supernatant from the cultured cells, or on a lysate or extract derived from the cells. (M) involves cultivating cells identified by the screening, and isolating the bioactive compound. A multiplicity of different nucleic acid sequences are introduced into a corresponding multiplicity of host cells or host cell populations so that a proportion of the cells are enabled to produce one or more compounds having the bioactivity, where the screening step leads to the identification of the cells. The multiplicity of transformed cells are enabled to produce a multiplicity of different bioactive compounds. (M) includes preparing multiplicity of different nucleic sequences by

providing a vector carrying one or more genes encoding proteins required for the biosynthesis of one or more bioactive substances, and genetically manipulating one or more of the genes on the vector to produce a multiplicity of vectors carrying different manipulated versions of the genes. A single nucleic acid sequence is introduced into a number of cells containing or providing respective different first enzymic activities so that at least some of the cells are enabled to produce respective different active compounds. The first enzymic activity is an activity that contributes to a secondary metabolic pathway. The first enzymic activity and/or the second enzymic activity comprises one or more respective activities selected from glycosylation, methylation, oxidation, polyketide synthesis, isomerization, ester formation, epimerization, decarboxylation, lactonization, acetylation or other acylation, amination, reduction, dehydration, deoxysugar synthesis, and starter unit synthesis (e.g. for polyketide synthesis). The second enzymatic activity leads to the production of a material which is a substrate for the first enzymic activity, which leads to the production of a bioactive material. The step of providing host cells includes providing a precursor cell and genetically manipulating it to produce the host cell. The introduction of nucleic acids employs a vector containing polyketide synthase (PKS) genes of a cluster or its derivatives, and the host cells are cells of a complementary but specifically prepared or selected host strain that possesses biosynthetic pathway enzymes which, when expressed together with the vector-encoded biosynthetic pathway genes, will give a desired bioactivity. The PKS genes of the vector have undergone genetic manipulation. The enzymes of the host cells have undergone genetic manipulation. Preferred Cells: (II) is selected from prokaryotic and eukaryotic organisms and cells of higher eukaryotes. The host cells are selected from prokaryotic fungal and mammalian cells. The host cells are actinomycete cells. The host cells are selected from Streptomyces coelicolor, S.avermitilis, S.griseofuscus, S.cinnamonensis, S.fradiae, S.eurythermus, S.longisporoflavus, S.hygroscopicus, S.lasaliensis, S.venezuelae, S.antibioticus, S.lividans, S.rimosus, S.albus, S.rochei, S.tsukubaensis, **Saccharopolyspora** erythraea, S.spinosa Micromonospora griseorubida, Actinoplanes sp., Amycolatopsis mediterranei and Nocardia sp..

USE - (M) is useful for producing a bioactive compound (selected from polyketides, nonribosomal peptides, mixed polyketide-nonribosomal peptides, fatty acids terpenes, alkaloids, aminoglycosides, shikimic acid derivatives, flavonoids, coumarins, polyglycosides, proteins polysaccharides, flavones and other flavonoids, nitrogen containing compounds such as indoles and pyrroles, anthraquinones, lignans, stilbenes, depsipeptides, peptides and nucleic acids, steroids and other hormones, preferably polyketide) having a known bioactivity (such as a pharmacological activity e.g. antibacterial, antifungal, anticancer, antiviral, motilide, insecticidal, antihelmintic, herbicidal, anticoccidal, anticoagulant, anti-inflammatory, antiprotozoal, antiplatelet, anti-hypertensive, antiproliferative, proliferative, neuroregenerative, hair growth promoting, anti-fibrotic, antimalarial, antiplasmodial, antiangiogenesis, anticholesterol, cytotoxic, protein inhibition, protein synthesis inhibition, protein activation or immunosuppressant activities) (claimed).

ADVANTAGE - (M) allows the direct in vivo modification of the polyketide synthase (PKS) products to give active compounds, therefore allowing direct screening of cells. (M) generates and expresses PKS/hybrid PKSs in a manner that does not require gene replacement for every desired change since this was or can be laborious and time consuming. (M) allows rapid detection of genetic constructs that have accumulated mutations or undergone recombination or deletion, a particular problem when using highly repetitive DNA as encountered in PKS or NRPS gene clusters. Screening transformants directly has the benefit of allowing better producing cells or colonies to be screened from poorer producers before fermenting to isolate the bioactive product. (M) allows a rapid biological screen of production levels from many engineered

transformants and helps screen out problematic strains. (M) allows preparation of a modified PKS or biosynthetic pathway, or a portion of a PKS or biosynthetic pathway on a single vector under the same promoter region. In (M), all the required changes to prepare the modified/heterologous PKS can be made in Escherichia coli prior to transfer to the host strain and subsequent screen. An advantage of (M) is that because transformants are screened for bioactivity this is an easy check that an engineered plasmid construct (or other vector) is correct and functional. This is a particular advantage in systems where the plasmid construct is extremely large or inherently unstable to recombination, where conventional methods of screening for plasmid fidelity are difficult or inconclusive. A further advantage of (M) is that it effectively avoids the need to conduct all the gene replacement experiments in pathways by double crossover, a process that can be time consuming and laborious, indeed in some strains, impossible, particularly in PKS regions.

EXAMPLE - Construction of **Saccharopolyspora** erythraea NRRL2338 JC2 (pHP)20) and screening it for production of novel bioactive erythromycins. Plasmid pHPO20 was used to transform S.erythraea NRRL2338 JC2 protoplasts using standard techniques. S.erythraea NRRL2338 JC2 was precisely deleted of the entire DEBS1+2+3 leaving the TE as a homology region for integration of PKS plasmids. The transformation mixture was plated onto R2T20 plates and recovered for 24 hours before overlaying with 40 microg/ml thiostrepton. Colonies were grown for approximately 7-10 days to allow **sporulation** before harvesting the spores and plating dilutions onto R2T20 agar containing 40 microg/ml thiostrepton and grown for 7 days at 30 degrees Centigrade. Individual thiostrepton resistant colonies were patched in 1 cm2 areas onto a large (25x25 cm) square R2T20 agar plate leaving reasonable space between each patch and grown for approximately 5 days to allow secondary metabolite production. At this stage small, plugs of agar were taken from each patch and placed at equally spaced intervals on a Luria Bertani (LB) plate that had been overlayed with a culture of the indicator organism M.luteus. A patch of JC2 was used as a control. LB plate was then placed at 4 degrees Centigrade for 4 hours to allow diffusion of natural products from plug to the LB agar before moving the plate 37 degrees Centigrade to allow growth of the screening organism. Individual plugs were screened by size of zone of inhibition, allowing screening of non-produces versus producer organisms but additionally screening for better producing colonies. To further verify the introduction of the correct construct producer-clones were tested for the presence of pHPO20 integrated into the TE region by Southern blot hybridization of their genomic DNA with DIG labeled DNA containing 1 kb MscI/AvrII fragment of rapamycin AT2. All the producer-clones tested appeared to contain a correctly integrated copy of pHPO20, as could be predicted by the formation of bioactive products. To verify the exact chemical nature of the active product identified by the screening method two tests were made. Firstly, utilizing the remainder of the replica plated colony used to produce the plug for the screening organism. A second plug was taken and extracted with 2x1 ml ethyl acetate that had been adjusted to pH 9 (ammonia). The solvent from these extracts was removed by evaporation and the residue analyzed by HPLC/mass spectroscopy (MS). A peak was observed with molecular mass m/z (M+H)+ = 690 for 6-desmethyl erythromycin D. Secondly, S.erythraea NRRL 2338 (pHPO20) was used to inoculate 5 ml TSB containing 5 microg/ml thiostrepton. After three days growth 1.5 ml of this culture was used to inoculate 30 ml of EryP medium containing 5 microg/ml thiostrepton in a 250 ml flask. The flask was incubated at 30 degrees Centigrade, 250 rpm for 6 days. At this time the supernatant was adjusted to pH 9 with ammonia and extracted twice with an equal volume of ethyl acetate. The solvent was removed by evaporation and the residue was analyzed by HPLC/MS. A peak was observed with molecular mass m/z (M+H)+ = 690 for 6-desmethyl erythromycin D. Further analysis showed the presence of products due to incomplete post PKS processing, B, C and A forms, and compounds due to product breakdown. Quantification of the peak

corresponding to 6-desmethyl erythromycin D indicated the product was produced at approximately 1 mg/l under the flask conditions used. (107 pages)

L5 ANSWER 2 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2000:748302 SCISEARCH
THE GENUINE ARTICLE: 358UV
TITLE: ssgA is essential for **sporulation** of Streptomyces coelicolor A3(2) and affects hyphal development by stimulating septum formation
AUTHOR: vanWezel G P (Reprint); vanderMeulen J; Kawamoto S; Luiten R G M; Koerten H K; Kraal B
CORPORATE SOURCE: LEIDEN UNIV, LEIDEN INST CHEM, DEPT BIOCHEM, POB 9502, NL-2300 RA LEIDEN, NETHERLANDS (Reprint); LEIDEN UNIV, MED CTR, CTR ELECTRON MICROSCOPY, NL-2300 RA LEIDEN, NETHERLANDS; DSM ANTIINFECT, NL-2600 MA DELFT, NETHERLANDS; NATL FOOD RES INST, TSUKUBA, IBARAKI 305, JAPAN
COUNTRY OF AUTHOR: NETHERLANDS; JAPAN
SOURCE: JOURNAL OF BACTERIOLOGY, (OCT 2000) Vol. 182, No. 20, pp. 5653-5662.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904.
ISSN: 0021-9193.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 42

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The role of ssgA in cell division and development of streptomycetes was analyzed. An ssgA null mutant of Streptomyces coelicolor produced aerial hyphae but failed to **sporulate**, and ssgA can therefore be regarded as a novel whi gene. In addition to the morphological changes, antibiotic production was also disturbed, with strongly reduced actinorhodin production. These defects could be complemented by plasmid-borne ssgA. In the wild-type strain, transcription of ssA was induced by nutritional shift-down and was shown to be linked to that of the upstream-located gene ssgR, which belongs to the family of iclR-type transcriptional regulator genes. Analysis of mycelium harvested from liquid-grown cultures by transmission electron microscopy showed that septum formation had strongly increased in ssgA-overexpressing strains in comparison to wild-type S. coelicolor and that spore-like compartments were produced at high frequency. Furthermore, the hyphae were significantly wider and contained irregular and often extremely thick septa. These data underline the important role for ssgA in Streptomyces cell division.

L5 ANSWER 3 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2001:293410 SCISEARCH
THE GENUINE ARTICLE: 418XQ
TITLE: Effects of increased and deregulated expression of cell division genes on the morphology and on antibiotic production of streptomycetes
AUTHOR: van Wezel G P (Reprint); van der Meulen J; Taal E; Koerten H; Kraal B
CORPORATE SOURCE: Leiden Univ, Leiden Inst Chem, Dept Biochem, POB 9502, NL-2300 RA Leiden, Netherlands (Reprint); Leiden Univ, Leiden Inst Chem, Dept Biochem, NL-2300 RA Leiden, Netherlands; Leiden Univ, Med Ctr, Ctr Electron Microscopy, NL-2300 RA Leiden, Netherlands
COUNTRY OF AUTHOR: Netherlands
SOURCE: ANTONIE VAN LEEUWENHOEK INTERNATIONAL JOURNAL OF GENERAL

AND MOLECULAR MICROBIOLOGY, (DEC 2000) Vol. 78, No. 3-4, pp. 269-276.
Publisher: KLUWER ACADEMIC PUBL, SPUIBOULEVARD 50, PO BOX 17, 3300 AA DORDRECHT, NETHERLANDS.
ISSN: 0003-6072.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 34
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB This paper describes the effects of increased expression of the cell division genes ftsZ, ftsQ, and ssgA on the development of both solid- and liquid-grown mycelium of Streptomyces coelicolor and Streptomyces lividans. Over-expression of ftsZ in S. coelicolor M145 inhibited aerial mycelium formation and blocked **sporulation**. Such deficient **sporulation** was also observed for the ftsZ mutant. Over-expression of ftsZ also inhibited morphological differentiation in S. lividans 1326, although aerial mycelium formation was less reduced. Furthermore, antibiotic production was increased in both strains, and in particular the otherwise dormant actinorhodin biosynthesis cluster of S. lividans was activated in liquid- and solid-grown cultures. No significant alterations were observed when the gene dosage of ftsQ was increased. Analysis by transmission electron microscopy of an S. coelicolor strain over-expressing ssgA showed that septum formation had strongly increased in comparison to wild-type S. coelicolor, showing that SsgA clearly influences Streptomyces cell division. The morphology of the hyphae was affected such that irregular septa were produced with a significantly wider diameter, thereby forming spore-like compartments. This suggests that ssgA can induce a process similar to submerged **sporulation** in Streptomyces strains that otherwise fail to do so. A working model is proposed for the regulation of septum formation and of submerged **sporulation**.

L5 ANSWER 4 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN DUPLICATE 1
ACCESSION NUMBER: 1998:219401 SCISEARCH
THE GENUINE ARTICLE: ZB053
TITLE: Use of reaction calorimetry to monitor and control microbial cultures producing industrially relevant secondary metabolites
AUTHOR: Voisard D (Reprint); Claivaz C; Menoud L; Marison I W; vonStockar U
CORPORATE SOURCE: ECOLE POLYTECH FED LAUSANNE, DC, INST CHEM ENGN & BIOENGN, CH-1015 LAUSANNE, SWITZERLAND (Reprint); CIBA SPECIALITES CHIM MONTHEY SA, CH-1870 MONTHEY, SWITZERLAND
COUNTRY OF AUTHOR: SWITZERLAND
SOURCE: THERMOCHIMICA ACTA, (26 JAN 1998) Vol. 309, No. 1-2, pp. 87-96.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0040-6031.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: PHYS
LANGUAGE: English
REFERENCE COUNT: 27
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In this article, bench-scale heat flux calorimetry is applied to monitor complex microbial systems producing secondary metabolites and having a theologically complex behavior. With such systems, biological metabolic activity can only be measured accurately if calorimetry is improved by on-line correction for stirring power variations using torque measurement.

First, a successful application to the production of the antibiotic erythromycin by **Saccharopolyspora** erythraea is presented. During a batch-culture study, it was shown that heat-flux calorimetry can

indicate the two main phases of the process, the exact moment of any substrate depletion, and the nature of the depleted substrate. A fed-batch strategy was set up to optimize erythromycin production. Cultures controlled by calorimetry with pulsed addition of the N-source during the trophophase and of the C-source during the idiophase allowed a higher productivity to be reached.

Second, an application of heat-flux calorimetry to the production of a bioinsecticide by the **sporulating** bacterium Bacillus sphaericus is presented. This project aims to understand, model and control the factors that effect growth, **sporulation** and insecticide production in a chemically defined medium. A batch-culture study has shown that calorimetry can be used to monitor the different phases of the process (growth, **sporulation**) and the different substrate depletions. The use of a control strategy to optimize production of the insecticidal protein is now under investigation. (C) 1998 Elsevier Science B.V.

L5 ANSWER 5 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 94:708275 SCISEARCH
THE GENUINE ARTICLE: PP947
TITLE: CHARACTERIZATION OF SPOOA HOMOLOGS IN DIVERSE BACILLUS AND CLOSTRIDIUM SPECIES IDENTIFIES A PROBABLE DNA-BINDING DOMAIN
AUTHOR: BROWN D P; GANOVARAEVA L; GREEN B D; WILKINSON S R; YOUNG M; YOUNGMAN P (Reprint)
CORPORATE SOURCE: UNIV GEORGIA, DEPT GENET, ATHENS, GA, 30602 (Reprint); UNIV GEORGIA, DEPT GENET, ATHENS, GA, 30602; UNIV WALES, INST BIOL SCI, ABERYSTWYTH SY23 3DA, DYFED, WALES
COUNTRY OF AUTHOR: USA; WALES
SOURCE: MOLECULAR MICROBIOLOGY, (NOV 1994) Vol. 14, No. 3, pp. 411-426.
ISSN: 0950-382X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 47

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB SpoOA is a phosphorylation-activated transcription factor of Bacillus subtilis. It is a member of the response regulator superfamily of bacterial signal transduction proteins and controls many of the changes in gene expression that occur during the transition into stationary phase and during the initiation of **sporulation**. To identify the domains of SpoOA most critical for determining its structural and functional features, presumptive homologues of the spoOA gene were characterized in a collection of eight Bacillus species and six Clostridium species representing phylogenetically diverse members of these genera. An alignment of the partial or complete DNA sequences of these homologues revealed three regions of especially high conservation in the effector domain. We speculate that the most highly conserved of these corresponds to the recognition helix of a putative helix-turn-helix motif, and, therefore, represents the actual DNA-contacting surface of the protein. In the case of homologues identified in Bacillus anthracis and Clostridium acetobutylicum and retrieved by polymerase chain reaction amplification, we confirmed by gene-disruption analysis that the homologue actually is required for initiation of **sporulation**. Apparent homologues of the B. subtilis spoIVB gene were also discovered immediately upstream from the spoOA homologues in all Bacillus and Clostridium species examined. The discovery of homologues of B. subtilis **sporulation** genes in these diverse species implies that the gene products required for specifying pathways of **sporulation**-specific gene activation and for determining key morphogenetic changes may be highly conserved and suggests that an approach similar to that undertaken here might be used as a general strategy to retrieve and compare their gene sequences.

Exhaustive efforts to detect a spo0A-like gene in non-endospore formers, including close relatives of Bacillus such as Listeria and Staphylococcus, were uniformly unsuccessful, suggesting that regulation of gene activity during the transition into stationary phase mediated by Spo0A-like proteins may be exclusive to the endospore-forming bacteria.

L5 ANSWER 6 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 92:405210 SCISEARCH
THE GENUINE ARTICLE: JB681
TITLE: THE EVOLUTIONARY ROLE OF SECONDARY METABOLITES - A REVIEW
AUTHOR: MAPLESTONE R A; STONE M J; WILLIAMS D H (Reprint)
CORPORATE SOURCE: UNIV CAMBRIDGE, CHEM LAB, LENSFIELD RD, CAMBRIDGE CB2 1EW, ENGLAND
COUNTRY OF AUTHOR: ENGLAND
SOURCE: GENE, (15 JUN 1992) Vol. 115, No. 1-2, pp. 151-157. ISSN: 0378-1119.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 33
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB It is argued that organisms have evolved the ability to biosynthesise secondary metabolites ('natural products') due to the selectional advantages they obtain as a result of the functions of the compounds. Pleiotropic switching, the simultaneous expression of **sporulation** and antibiotic biosynthesis genes in Streptomyces, is interpreted in terms of the defense roles of antibiotics. The clustering together of antibiotic biosynthesis, regulation, and resistance genes. and in particular the staggering complexity shown in the case of the gene cluster for erythromycin A biosynthesis, implies that these genes have been selected as a group and that the antibiotics function in antagonistic capacities in nature.

L5 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 2
ACCESSION NUMBER: 1990:192749 CAPLUS
DOCUMENT NUMBER: 112:192749
TITLE: Cloning, characterization, and heterologous expression of the **Saccharopolyspora** erythraea (Streptomyces erythraeus) gene encoding an EF-hand calcium-binding protein
AUTHOR(S): Swan, David G.; Cortes, Jesus; Hale, Richard S.; Leadlay, Peter F.
CORPORATE SOURCE: Dep. Biochem., Univ. Cambridge, Cambridge, CB2 1QW, UK
SOURCE: Journal of Bacteriology (1989), 171(10), 5614-19 CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The regulatory effects of Ca2+ in eukaryotic cells are mostly mediated by a superfamily of Ca2+-binding proteins (CABs) that contain one or more characteristic Ca2+-binding structural motifs, referred to as EF hands. The structural gene for a authentic EF-hand CAB was cloned and sequenced from the spore-forming gram-pos. bacterium **Saccharopolyspora** erythraea (formerly Streptomyces erythraeus). When the gene was introduced into Streptomyces lividans on the high-copy plasmid vector pIJ702, CAB was found to be expressed at higher levels than in S. erythraea, with no apparent effects on either growth or **sporulation**. A more convenient expression system for CAB was obtained by introducing an NdeI site at the initiation codon by using oligonucleotide-directed mutagenesis and placing the gene in the expression vector pT7-7 in Escherichia coli. In this system, CAB was efficiently expressed at levels up to 20 to 30% of total cell protein. When purified to homogeneity from either E. coli or Streptomyces lividans, CAB was found to be identical to the protein previously obtained from S.

erythraea.

L5 ANSWER 8 OF 9 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1989-14266 BIOTECHDS
TITLE: Identification of a gene required for the terminal step in erythromycin-A biosynthesis in **Saccharopolyspora** erythraea (Streptomyces erythreus); mutant isolation
AUTHOR: Weber J M; Schoner B; *Losick R
CORPORATE SOURCE: Lilly
LOCATION: Department of Cellular and Developmental Biology, The Biological Laboratories, Harvard University, 16 Divinity Ave., Cambridge, MA 02138, USA.
SOURCE: Gene; (1989) 75, 2, 235-41
CODEN: GENED6
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A transcription unit was identified in the ermE region of the chromosome of the erythromycin-producing bacterium **Saccharopolyspora** erythraea UW110 (Streptomyces erythreus), which was briefly switched on at the onset of macrolide production. The location and orientation of the sequence was determined by S1 nuclease mapping experiments. Disruption of the transcription unit, eryG, by insertion of an integrational plasmid pMW11 vector, caused a block at the terminal step in the biosynthesis of erythromycin, the conversion of erythromycin-C to A by O-methylation, catalyzed by O-methyltransferase. The recombinant strain was not measurably different than the parent strain in growth and **sporulation** properties or morphology, but was impaired in antibiotic production. Thus the erythromycin biosynthesis genes were clustered in the vicinity of the erythromycin-resistance ermE gene, and the gene product localized was involved in a terminal step in erythromycin biosynthesis. (12 ref)

L5 ANSWER 9 OF 9 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
DUPLICATE 3
ACCESSION NUMBER: 1975:228522 BIOSIS
DOCUMENT NUMBER: PREV197560058518; BA60:58518
TITLE: A NOVEL ACTINOMYCETE FROM SUGARCANE BAGASSE **SACCHAROPOLYSPORA**-HIRSUTA NEW-GENUS NEW-SPECIES.
AUTHOR(S): LACEY J; GOODFELLOW M
SOURCE: Journal of General Microbiology, (1975) Vol. 88, No. 1, pp. 75-85.
CODEN: JGMIAN. ISSN: 0022-1287.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: Unavailable

=> d his